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Short communication

Simultaneous determination of qualitatively important components in green tea infusions using capillary electrophoresis

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Abstract

Capillary zone electrophoresis (CZE) was adapted for the simultaneous determination of major components in green tea infusions. Separation was achieved using a fused-silica capillary column with a borax buffer at pH 8.0 and UV detection at 200 nm. The components analyzed were (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate, (+)-catechin, caffeine, theanine and ascorbic acid. The concentrations of these components were significantly different among various kinds of teas. CZE can be a very useful tool to estimate the quality and taste of green tea.

Keywords: Tea; Food analysis; Catechins; Caffeine; Theanine; Ascorbic acid

1. Introduction

In Japan green teas of various grades are sold at markets, and the prices range from 100 to several thousand yen per 100 g. The quality of green tea has been evaluated by organoleptic test of trained specialists, but recently the relationship between tea quality and its chemical components, free amino acids, catechins, caffeine and ascorbic acid, is being made clear [1]. Free amino acids, the key quality components of tea, are contained in higher amounts in higher grade teas. Among free amino acids, the content of theanine (1-glutamyl-γ-ethylamide), which is the amino acid of the highest amount, shows the highest correlation to the price of green tea [2]. Catechins, other major components of tea, also contribute to the astringent and bitter taste of tea. These days prophylactic properties of tea catechins are also attracting attention [3]. Caffeine is another important bitter component, which is known as a famous stimulant. Green tea contains abundant amounts of ascorbic acid, which is an antioxidant and acts as vitamin C. These days canned tea drink has become popular in Japan. Ascorbic acid is also added to canned tea drink to maintain its quality [4].

Many analytical methods have been developed to measure these important tea components in order to estimate the quality and taste of tea. Using high-performance liquid chromatography (HPLC), several catechins and caffeine can be separated [4,5], but no chemical method has been reported yet by which theanine and ascorbic acid can be measured simultaneously with catechins and caffeine in a single run. Thus, these important components must be analyzed separately by using several different methods. Can-

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calon and Bryan separated amino acids, polyphenols and ascorbic acid in citrus juice simultaneously by capillary zone electrophoresis (CZE) [6]. We report a CZE method for the simultaneous determination of five catechins, caffeine, theanine and ascorbic acid in green tea infusions.

2. Experimental

2.1. Reagents and tea samples

Five major tea catechins, (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC) and (+)-catechin (C) were kind gifts from Mitsui Norin (Tokyo, Japan). Theanine was purchased from Tokyo Kasei (Tokyo, Japan). Other reagents used were analytical grade.

Sencha teas of first flush and second flush were produced in our institute and stored under −20°C. Gyokuro tea was a gift from Saga Prefectural Tea Experiment Station and was stored in refrigerator. Canned tea drink was purchased from a market.

2.2. Instruments

Capillary electrophoresis system P/ACE 5000 with UV detector (Beckman Instruments, Fullerton, CA, USA) was used. The silica capillary tubing employed for analysis was uncoated, with an internal diameter of 50 μm and a length of 77 cm (70 cm from autosampler to detector).

2.3. Analytical conditions

Electropherograms were obtained with 20 m*M* borax buffer, prepared with deionized water (Milli-Q, Millipore) and adjusted to the appropriate pH with hydrochloric acid. The applied potential was 30 kV and detection was performed with a UV detector using 200-nm filter. The temperature of the capillary was maintained at 23°C. The standard mixture was prepared by dissolving five catechins, theanine, ascorbic acid and caffeine in 0.1% metaphosphoric acid. The concentration of each component was 50 mg l^{-1}. Tea infusion was prepared by the extraction from 3 g of tea leaves with 180 ml of boiling water for 5 min. The infusion filtered with No. 2 filter paper (Advantec, Tokyo, Japan) was diluted 10 times with 0.1% metaphosphoric acid. After passing through the 0.45-μm membrane filter, the filtrates were used as samples. Canned tea drink was diluted five times with 0.1% metaphosphoric acid and passed through the membrane filter and the filtrate was used as a sample. Sample was injected into the capillary by N_2 pressure for 5 s. After each separation, the capillary was rinsed sequentially with distilled water, 0.1 *M* HCl, distilled water, 0.1 *M* NaOH and buffer, between successive electrophoretic runs. The total time for rinsing was about 7 min.

3. Results and discussion

The λ_{max} for caffeine, ascorbic acid and catechins are 272 nm, 266 nm and around 280 nm, respectively, whereas there is no λ_{max} for theanine above 200 nm. We chose 200 nm as the detection wavelength to determine the concentration of theanine together with other components.

The standard sample mixture was separated using 20 m*M* borax buffers of the pH range of 7.6–9.0 as running buffers (Fig. 1). At pH 9.0 these eight components seemed to be very finely separated

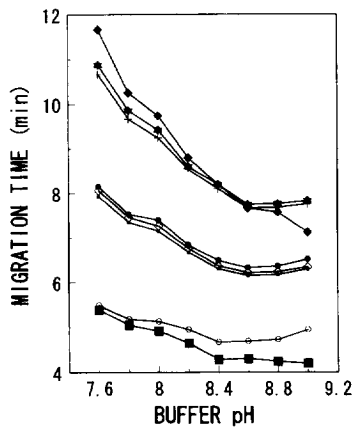

Fig. 1. The effect of pH of borax buffer on the migration time of eight tea compounds. ■=caffeine; ○=theanine; ■=(−)-epigallocatechin; ◇=(−)-epicatechin; ●=(+)-catechin; +=(−)-epigallocatechin gallate; ★=(−)-epicatechin gallate; ♦=ascorbic acid. Experimental conditions were: fused-silica capillary, total length 77 cm, length to detector 70 cm; buffer 20 m*M* borax; voltage 30 kV; detection 200 nm; temperature 23°C; injection time 5.0 s.

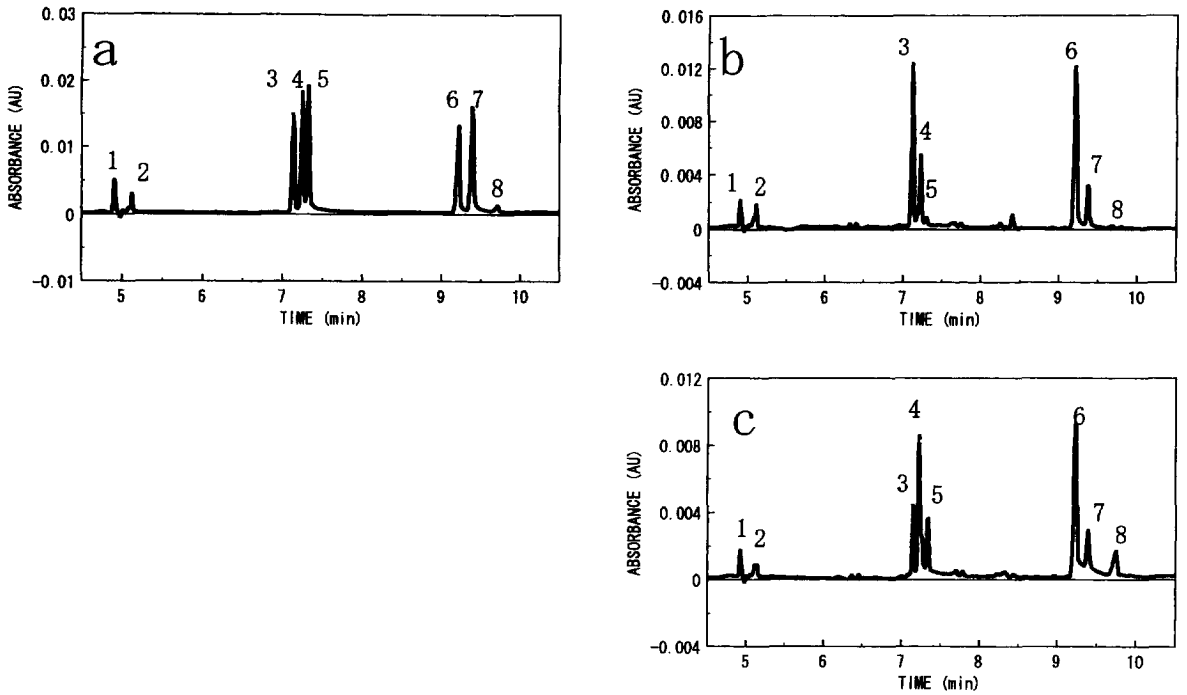

Fig. 2. Electropherograms of standard mixture (a), tea infusion of first flush Sencha (b) and canned tea drink (c). The concentrations of eight compounds were 50 mg l^{-1} each in (a). The dilution rate was ten and five times in (b) and (c), respectively. Peaks: 1=caffeine; 2=theanine; 3=(−)-epigallocatechin; 4=(−)-epicatechin; 5=(+)-catechin; 6=(−)-epigallocatechin gallate; 7=(−)-epicatechin gallate; 8=ascorbic acid. Experimental conditions were: fused-silica capillary, total length 77 cm, length to detector 70 cm; buffer 20 m*M* borax (pH 8.0); voltage 30 kV; detection 200 nm; temperature 23°C; injection time 5.0 s.

within 9 min. However, catechins are known to be unstable under alkaline conditions, and at higher pH values of buffer solution the tailing of the peaks became significant. At pH values lower than 7.8, the separation of theanine is not satisfactory. Finally we chose pH 8.0. In these conditions eight major components of green tea can be separated within 10 min. The electropherograms of the standard mixture and some authentic tea infusions are shown in Fig. 2.

Various tea infusions were analyzed the above mentioned conditions (Table 1). As for Sencha teas, it has been reported that first flush tea is of higher

Table 1
Concentrations of eight components in various tea infusions

	Concentration (mg l^{-1})			
	Sencha (first flush)	Sencha (second flush)	Gyokuro	Canned tea drink
EGCG	537.1 (7.1)	516.3 (2.9)	325.3 (4.4)	197.8 (2.7)
ECG	92.8 (1.7)	127.7 (1.7)	63.2 (3.0)	43.5 (1.0)
EGC	453.2 (4.8)	367.3 (2.3)	146.4 (3.3)	26.8 (0.7)
EC	153.8 (1.3)	115.9 (2.1)	51.3 (0.6)	62.8 (0.9)
C	18.3 (1.1)	17.0 (1.0)	6.1 (0.5)	110.2 (3.7)
Caffeine	208.1 (2.2)	161.5 (7.3)	261.6 (1.8)	82.9 (2.9)
Theanine	234.5 (5.5)	43.0 (0.7)	257.2 (1.0)	26.8 (0.7)
Ascorbic acid	80.1 (5.7)	36.4 (1.4)	ND	507.1 (5.6)

Numbers in parentheses=standard deviation (n=3). ND: less than 10 mg l^{-1}. EGCG: (−)-epigallocatechin gallate; ECG: (−)-epicatechin gallate; EGC: (−)-epigallocatechin; EC: (−)-epicatechin; C: (+)-catechin.

quality and contains higher amounts of theanine [7]. In our results, the infusion of first flush tea showed a higher concentration of theanine than that of the second flush. Gyokuro is a special high grade tea, which is reported to contain higher amounts of theanine and caffeine, and less catechins and ascorbic acid than Sencha tea [1]. The results of our experiment were consistent with that. At the process of manufacturing canned drink, ascorbic acid is added to maintain the quality, and the composition of catechins is changed by the high-temperature treatment to kill the microorganisms [4]. In our experiments, much higher amounts of ascorbic acid and (+)-catechin were observed in canned tea drink than in the infusions from tea leaves.

In our conditions, the time for the analysis is only 11 min and it needs less than 8 min for rinsing. Several HPLC methods to separate catechins and caffeine have been reported, but the time needed for one sample is more than 20 min [4,5] and neither theanine nor ascorbic acid could be simultaneously analyzed with catechins. This CZE method is more suitable than HPLC methods to estimate the quality and taste of green tea, in terms of a shorter analytical time and a simultaneous determination of more components in a single run.

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